

**SCREENING OF ALGAL AND CYANOBACTERIAL STRAINS FOR OPTIMAL
BIOMASS PRODUCTION AND NUTRIENT RECOVERY FROM DAIRY MANURE-
BASED ANAEROBIC DIGESTION EFFLUENT**

by

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Abstract

This research screened 4 cyanobacteria strains and 16 algae strains, including both isolated environmental species and strains purchased from the Culture Collection of Algae at the University of Texas at Austin (UTEX), for their ability to grow on anaerobic digestion effluent (ADE). The 4 most robust strains, based on growth rates and biomass production, were more closely investigated for their capacity to extract nitrogen (N) and phosphorus (P) from deionized water supplemented with 1, 5, and 10% ADE. According to the results, the decrease in concentration of total nitrogen (TN) varied between the strains examined. The greatest consumption of TN for 1% ADE was 88% by the wastewater algae strain AS-A1, while the least change was 26% by strain UTEX B SP23. However, the removal of total phosphorus (TP) was nearly 70% for all measured strains at 5 and 10% ADE. The maximum biomass production was in the range of 354-366 mg/L by UTEX 1237 in 10% ADE. Biological assimilation was speculated as the main reason of nitrogen and phosphorus removal as control experiments absent of algae showed minimal nutrient concentration change. In short, anaerobic digestion effluent is an economical and beneficial resource that can be used to cultivate microalgae and cyanobacteria. Selecting the combinations of robust strains and suitable effluent concentrations that yield optimal biomass and uptake of nutrients is a critical step before upscaling to industrial applications.

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Introduction

The interest in adopting renewable and sustainable energy has been driven significantly by the world's rising energy demands and the increased awareness of the detrimental impacts caused by continued use of traditional fossil fuels (Wenguang Zhou et al., 2011). Biofuels produced from algal cells (e.g. biodiesel) have been investigated as an alternative energy source to fossil fuels (Chisti, 2008). There are multiple advantages to using microalgae as a source of biofuel including higher productivities when compared with terrestrial plants, potential land saving by non-arable cultivation, and the convenience of reusing industrial carbon dioxide (Bohutskyi and Bouwer, 2015). Furthermore, whole algae and cyanobacteria cells and lipid extracted cells can be used as feedstock material for anaerobic digestion. However, cultivation of these organisms requires large amounts of water with intensive supplementation of nutrients, particularly nitrogen (N) and phosphorus (P). More advanced cultivation methods need to be developed to maximize growth rates to eventually achieve efficient and reliable industrial algal and cyanobacterial production processes.

Currently, biofuels are being discussed in relation to its economic applicability and environmental advantages in the context of livestock farms. Traditional cultivation techniques utilizing freshwater is expensive because of the required additional nutrient supplementation, but also wasteful as valuable water resources are consumed (Pate et al., 2011). Thus, wastewater as an alternative low-cost source of nutrients and water could largely enhance the sustainability of microbially derived biofuels. The growth in the number of operating anaerobic digesters on livestock farms are shown in Figure 1. Although the total numbers are lower than

in Germany, for instance, there is an upward trend in the use of digesters on farms in the United States. Anaerobic digestion effluent (ADE) serves as a potential growth media for algae and cyanobacteria based on its typically high concentrations of nitrogen and phosphorus (Lin Shi et al., 2018)

Since growing microalgae and cyanobacteria on farm wastewater to produce biofuels has the benefit of removing nutrients, it can therefore be considered as a wastewater treatment biotechnology. The overloading of watersheds with nutrients via discharge of untreated farm wastewater leads to the deterioration of aquatic ecosystems through eutrophication (Moree et al. 2013, Bricker et al., 2008). Moreover, traditional wastewater treatment technologies for removing nutrients (e.g. enhanced nutrient removal, ENR) have high capital and operational costs and are therefore not typically enacted on livestock farms. Furthermore, microalgal and cyanobacterial cultivation has an additional benefit of photosynthetically increasing the dissolved oxygen (DO) in the wastewater during the nutrient recovery process. The effluent from these cultivation systems (e.g. algal turf scrubber lagoons) will be applied to farms soils which will benefit from higher DO concentrations.

Although many of the feasibilities of algal cultivation have been tested in different wastewaters, the performance of algal cultivation is still often limited by inadequate growth and culture robustness (Bohutskyi and Bouwer, 2015). As a result, it is essential to screen, identify and characterize strains that perform better than competitive microorganisms that exist in wastewater in order to maintain a reliable productivity and provide a predictable quality of biomass. Previous studies provide valuable data related to growth rates and biomass production, nutrient removal efficiency, and biofuel productivity based on the fraction of lipids in the

biomass under various conditions, for instance, in high carbon dioxide concentrations (Li et al. 2011; Zhou et al. 2012) and low temperature (Abdelaziz et al. 2014). However, it is crucial to extrapolate those laboratory results into full-scale wastewater treatment modestly since most of them used sterilized wastewater as cultivated resources, which can be a tough problem at large scale. Moreover, unsterilized livestock ADE may contain microorganisms that are harmful to microalgae and cyanobacteria, such as protozoa, bacteria, fungi and viruses. Thus, it is critical for us to screen for strains that can perform well even in highly competitive environments.

Livestock farm ADE may be an ideal resource for cultivation since it contains abundant nutrients for algal and cyanobacterial growth and could be cost effective for farmers. In this study, we not only screened potential algae strains by using unsterilized ADE, but also tested the biomass production and efficiency of nutrient recovery of selected strains in gradient concentration of anaerobic digestion effluent centrifugate.

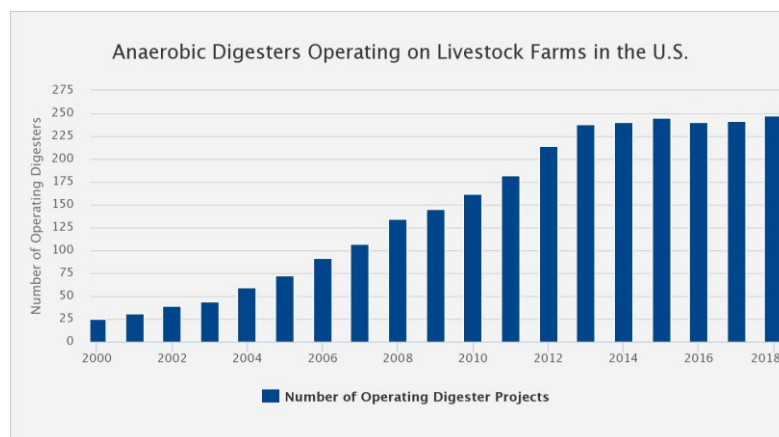


Figure 1. The growth in the number of operating digester systems on livestock farms in the U.S. from 2000 to 2018 (Source: AgSTAR Livestock Anaerobic Digester Database).

Methods and materials

Algae strains and growth medium

The algae and cyanobacteria strains used in this study are listed in Table 1. Many were purchased from the Culture Collection of Algae at the University of Texas at Austin (UTEX). *Chlorella*, *Scenedesmus*, and *Chlamydomonas* strains were screened because of their successful biomass production in municipal wastewater (Bohutskyi and Bouwer, 2015). Cyanobacteria were purchased because of their generally fast growth rates. Environmental strains were collected and isolated by serial dilution culturing on BG11 agar petri dishes. Strain ASA1 was isolated from activated sludge collected from the Back River Wastewater Treatment Plant (BRWWTP) (Baltimore, MD, USA). All of the strains as shown in Table 1 were maintained in BG11 solid and liquid medium prior to each experiment. The recipe of BG11 media is shown below in Table. 2. To acquire cells from the stationary phase, algal inoculum cultures were grown in 100 mL conical flasks with 50 mL sterilized BG11 media under fluorescent lighting (3.55 Klux, 16/8-hour light/dark photoperiod) at 28.6 ± 2 °C for 5-7 days and mixed at 250 rpm with 20 mm stir bars. Light intensity was obtained by using an Extech light meter (Model EA30 EasyView™ Wide Range Light Meter by Extech Instruments, Nashua, NH, USA).

Table 1. List of algae and cyanobacteria strains screened in this study. Strains that are bolded were subsequently investigated for nutrient recovery and biomass production.

Strain	Species/Environment
UTEX 1584	<i>Scenedesmus parisiensis</i>
UTEX 2532	<i>Scenedesmus subspicatus</i>
UTEX 1237	<i>Scenedesmus dimorphus</i>
UTEX 74	<i>Scenedesmus naegeli</i>
UTEX 2714	<i>Chlorella vulgaris</i>
UTEX 260	<i>Chlorella sorokiniana</i>
UTEX 395	<i>Chlorella vulgaris</i>
UTEX 2911	<i>Chlorella saccharophila</i>
UTEX 214	<i>Chlamydomonas pseudococcum</i>
UTEX 969	<i>Chlamydomonas applanata</i>
UTEX LB 2386	<i>Microcystis aeruginosa</i>
UTEX 2470	<i>Synechocystis</i> sp.
UTEX B SP23	<i>Aphanocapsa</i> sp.
UTEX LB 1902	<i>Merismopedia</i> sp.
AP-L	Aquaponics System, Cylburn Arboretum
FFW	Aquaponics System, Cylburn Arboretum
PE-01	Primary Effluent, BRWWTP
PE-Y	Primary Effluent, BRWWTP
AS-A1	Activated Sludge, BRWWTP
AS-A2	Activated Sludge, BRWWTP

Table 2. The recipe of Blue-Green Medium per liter. Adjusted pH to 7.1 with 1M NaOH or HCl prior to autoclaving.

Chemical compounds	Concentration (g/L)
NaNO ₃	1.5
K ₂ HPO ₄	4×10 ⁻²
MgSO ₄ ·7H ₂ O	7.5×10 ⁻²
CaCl ₂ ·2H ₂ O	3.6×10 ⁻²
Citric acid	6×10 ⁻³
Ammonium ferric citrate green	6×10 ⁻³
EDTANa ₂	1×10 ⁻³
Na ₂ CO ₃	2×10 ⁻²
H ₃ BO ₃	2.86×10 ⁻³
MnCl ₂ ·4H ₂ O	1.81×10 ⁻³
ZnSO ₄ ·7H ₂ O	2.2×10 ⁻⁴
Na ₂ MoO ₄ ·2H ₂ O	3.9×10 ⁻⁴
CuSO ₄ ·5H ₂ O	8×10 ⁻⁵
Co(NO ₃) ₂ ·6H ₂ O	5×10 ⁻⁵

Fluorescence microscope

Samples of each algae strains were collected from the final day of cultivation and observed using a fluorescence microscope (AxioObserverZ1/Apotome2) at the Johns Hopkins Integrated Imaging Center. These images demonstrate the conditions of each algae strain at the end of the experiment.

Cultivation experiments and anaerobic digestion effluent

Anaerobic digestion effluent (ADE) was obtained from a laboratory-scale (2 Liter) anaerobic digester operating semi-continuously in Prof. Edward Bouwer's lab at Johns Hopkins University, on dairy manure provided by Dr. Jactone Ogejo from Virginia Polytechnic Institute and State University. The mesophilic digester (35C) had a hydraulic retention time of 17 days, and a dairy manure organic loading rate of 1 gVS/L_{reactor}/day. During the initial screening process, two concentrations (1% and 5%) of ADE were investigated to identify optimal strains based on biomass production. ADE samples for 1% and 5% initial screening experiments were collected and diluted on Aug. 15th, 2018 and Dec. 9th, 2018, respectively. Three concentrations (1%, 5% and 10%) of anaerobic digestion effluent centrifugate were subsequently involved to test the efficiency of nutrient recovery of selected strains, as well as reconfirm the biomass production ability. ADE that was used for growing selected strains was a mixture of effluent samples that were collected and frozen between Oct. 4th, 2018 to Oct. 10th, 2018. Selected samples of ADE were centrifuged at 3900 rpm at 25 °C for 10 mins followed by supernatant collection. Microalgae strains were grown in 250 mL conical flasks with 100 mL total volume of solution under fluorescent light (3.54 Klux, 16/8-hour light/dark photoperiod) and aerated with 0.45 μ m filtered 1% CO₂ gas at 28.6 \pm 2 °C for 7-10 days. Follow-up experiments were conducted in biological duplicates.

Algal and cyanobacterial growth rate determination

Optical density (OD) of each sample of algal culture was measured using an ultraviolet

spectrophotometer (UV-1800, Shimadzu) at 680 nm to detect chlorophyll. The corresponding first-order growth rate (k_1 , day⁻¹) of each strain was calculated using the following formulas:

$$OD_{strain} = OD_i - OD_{control}$$

$$\ln(OD_{strain}^t) = k_1 t + \ln(OD_{strain}^0)$$

OD_i represents the optical density of the inoculated ADE sample, while $OD_{control}$ means the optical density of uninoculated ADE sample (positive control). Related linear regression statistics were calculated using Excel.

Biomass determination

Biomass for each algal strain was measured on the final day of cultivation. Samples were transferred from conical flasks to centrifuge tubes and freeze-dried for 40 hours using a Labconco Freeze Dry System.

Nutrient measurements and removal efficiencies

Total nitrogen (TN) was measured using the Shimadzu Total Nitrogen unit (TNM-L) with potassium nitrate as the analytical standard. Nitrate (NO_3^-), ammonia (NH_3), total phosphorus (TP), and phosphate (PO_4^{3-}) were measured using Hach TNT Nutrient Kits. Removal efficiency measured in ADE amended solutions before and after culturing are calculated using the following equation:

$$\text{RE, \%} = \left[\frac{C_0 - C_t}{C_0} \right] \times 100\%$$

C_0 represents the nutrient concentration (mg/L) of samples at day 0, while C_t represents the nutrient concentration (mg/L) at day t.

Results

Screening

A total of 16 algal strains and 4 cyanobacteria strains took part in the primary screening process, and their time series growth curves are shown below in Figure 2 and Figure 3. Growth rates (k_1 , day^{-1}) from each strain are shown in Figure 4, and total biomass produced is displayed in Figure 5. As we can see from Figure 2 and 3, 12 of 20 strains have significantly higher optical density when they were cultivated in 5% compare to 1% ADE after around 4 days cultivation, while 6 strains only have slightly higher optical density when they were cultivated in 5% than in 1% ADE and 3 strains kept having lower optical density in 5% ADE. The relationship between optical density and time of each strain seems to be linear and exponential in 1% and 5% ADE, respectively, even though the optical density of algae strains that were grown in 5% ADE are not monotonically increasing with time. As shown in Figure 2 and 3, there are 10 strains that have 1.64 to 4.39 times higher growth rate in 5% than 1% ADE whereas 9 of the other 10 strains have 0 growth rate in 5% ADE and 1 of 9 strains has 0 growth rate in 1% ADE. Based on the data of biomass (Figure 4), all of algae strains yield a higher production of biomass in 5% ADE compared with 1% ADE except for 3 strains (UTES 625, UTEX 393 and UTEX 1907) that did not successfully cultivate in 5% ADE. Biomass production of UTEX 2386 in 5% ADE is 33.1 times of the biomass production in 1% ADE while most of the algal strains' biomass production in 5% ADE are around 3.04 to 11.6 times of production in 1% ADE.

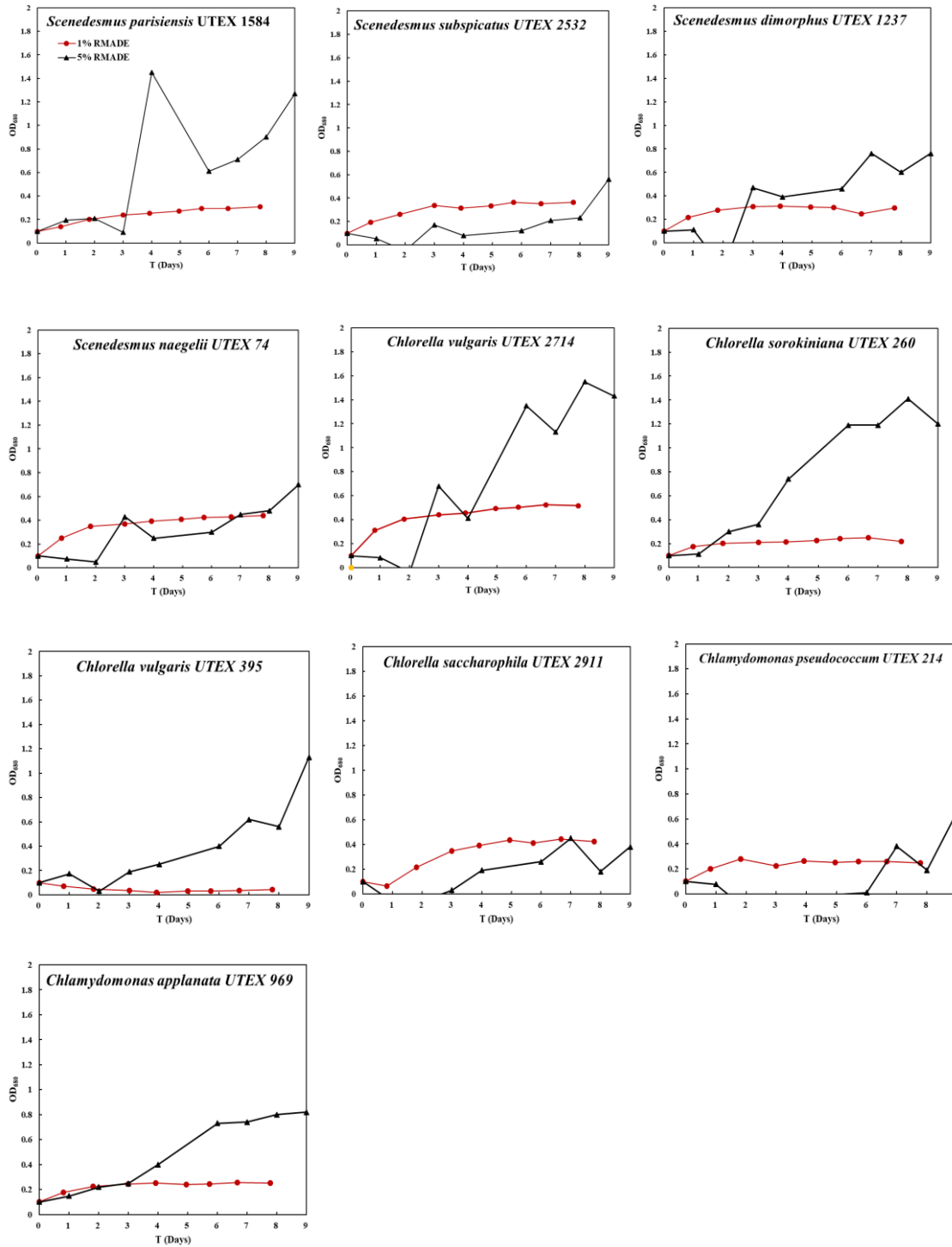


Figure 2. Growth curve of 10 algae strains based on corresponding optical density versus cultivation time. Red circle and black triangle corresponding to optical density of algae strains in 1% and 5% ADE, respectively.

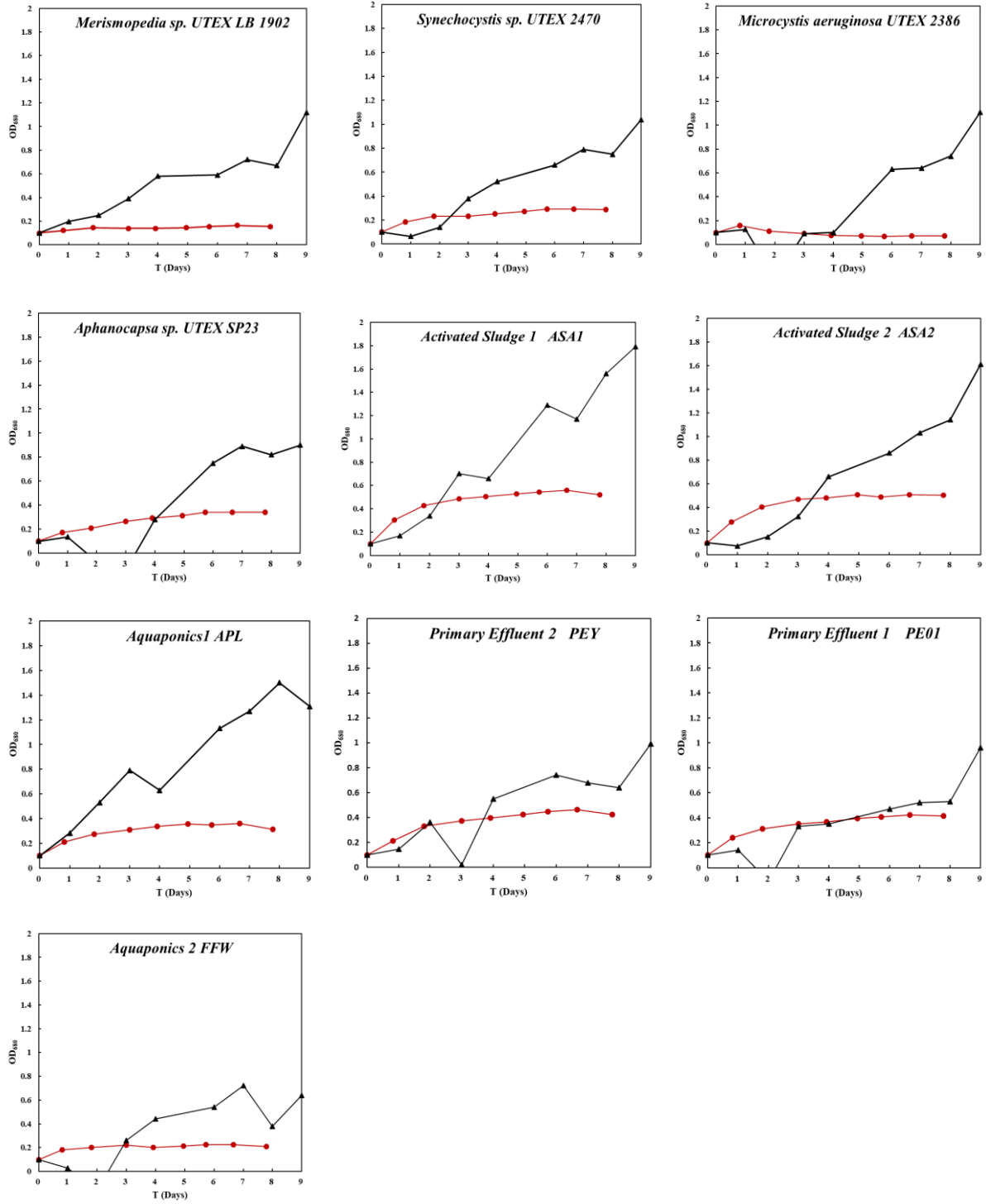


Figure 3. Growth curve of the rest of 10 algae strains based on corresponding optical density versus cultivation time. Red circle and black triangle corresponding to optical density of algae strains in 1% and 5% ADE, respectively.

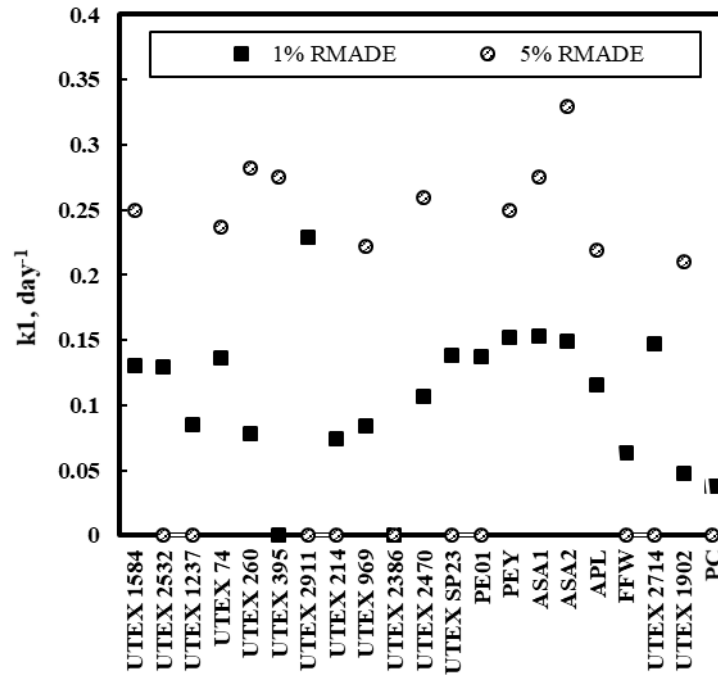


Figure 4. k_1 (day⁻¹) of each strain under two different concentrations of dairy manure ADE. Red points and black points represent the growth rate of algae in 5% ADE and 1% ADE, respectively.

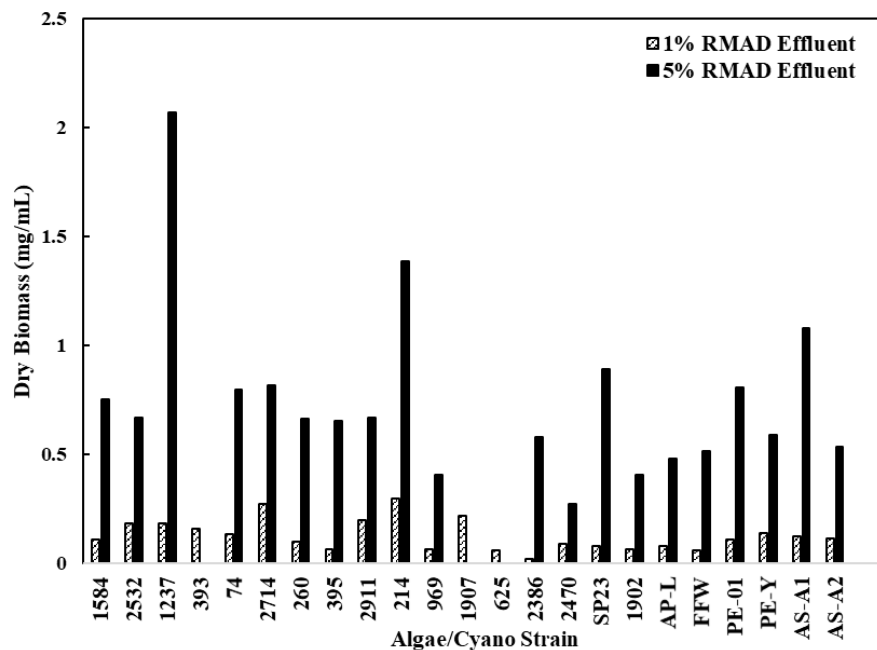


Figure 5. Dry biomass (mg/mL) of each strain that was cultivated for 8 days under two different concentrations of ADE.

Selected strains cultivation

Four strains including UTEX 214, UTEX 1237, UTEX SP23 and ASA1 were selected based on their high performance as biomass producers during cultivation in 1% and 5% uncentrifuged ADE (see Figure 5). They were recultured for subsequent investigations in 1%, 5% and 10% centrifugate ADE. Nutrient characteristics of the centrifugate ADE are listed in Table 3. According to Figure 6, ASA1 and UTEX 1237 both had the highest daily optical density in 10% ADE and lowest optical density in 1% ADE, UTEX SP23 also followed that pattern except for the last two cultivation days. Meanwhile, UTEX 214 did not have a significant difference in optical density between any concentration of ADE. Indeed, ASA1 has the highest optical density while 214 has the lowest optical density among the four strains and three concentrations except for the fourth day's optical density of UTEX 1237 in 1% ADE which has a large standard deviation. In addition, UTEX SP23 showed a similar growth curve with other algae strains in 1% and 5% ADE but different in 10% ADE. The optical density started to decrease after 5 days while the other strains' optical density continually increased. Concerning their biomass producing performance (Figure 5), all four strains produced the highest biomass in 10% ADE and the lowest biomass in 1% ADE. Beyond that, UTEX 1237 has the highest biomass while UTEX SP23 has the lowest biomass in all ADE concentration at the end of cultivation.

Table 3. Nutrient characteristics of dairy manure ADE.

Parameter	Total Nitrogen	Nitrate	Ammonia	Total phosphorus	Active Phosphate
Concentration (mg/L-N, or mg/L-P)	335.5±5.7	27.6±2.8	223.8	21.4±6.4	31.47±3.8

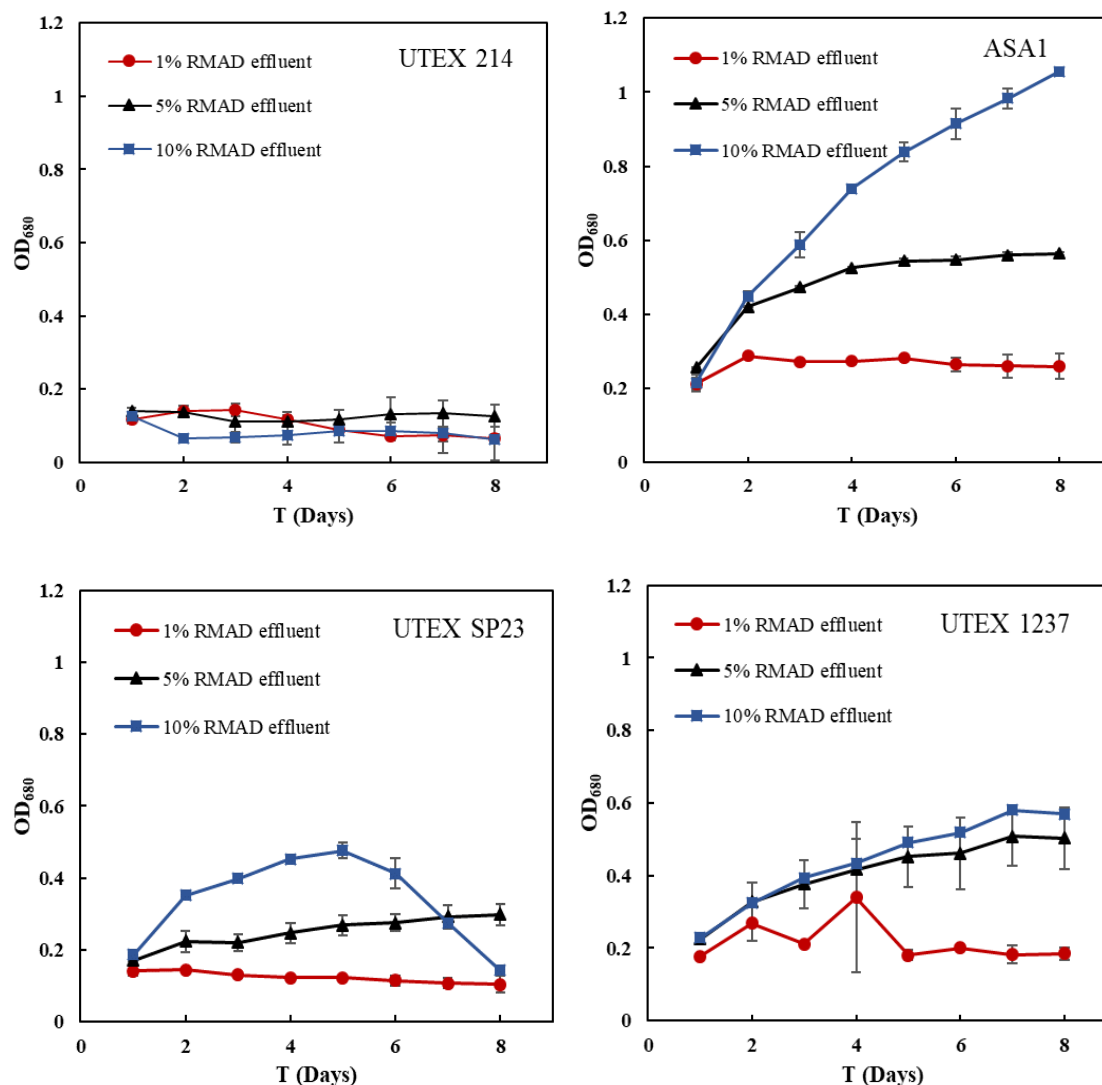


Figure 6. Optical density-based growth curves of four selected strains. Red circle, black triangle and blue square correspond to 1%, 5% and 10% ADE, respectively. Error bars were calculated as the standard deviation of biological duplicates.

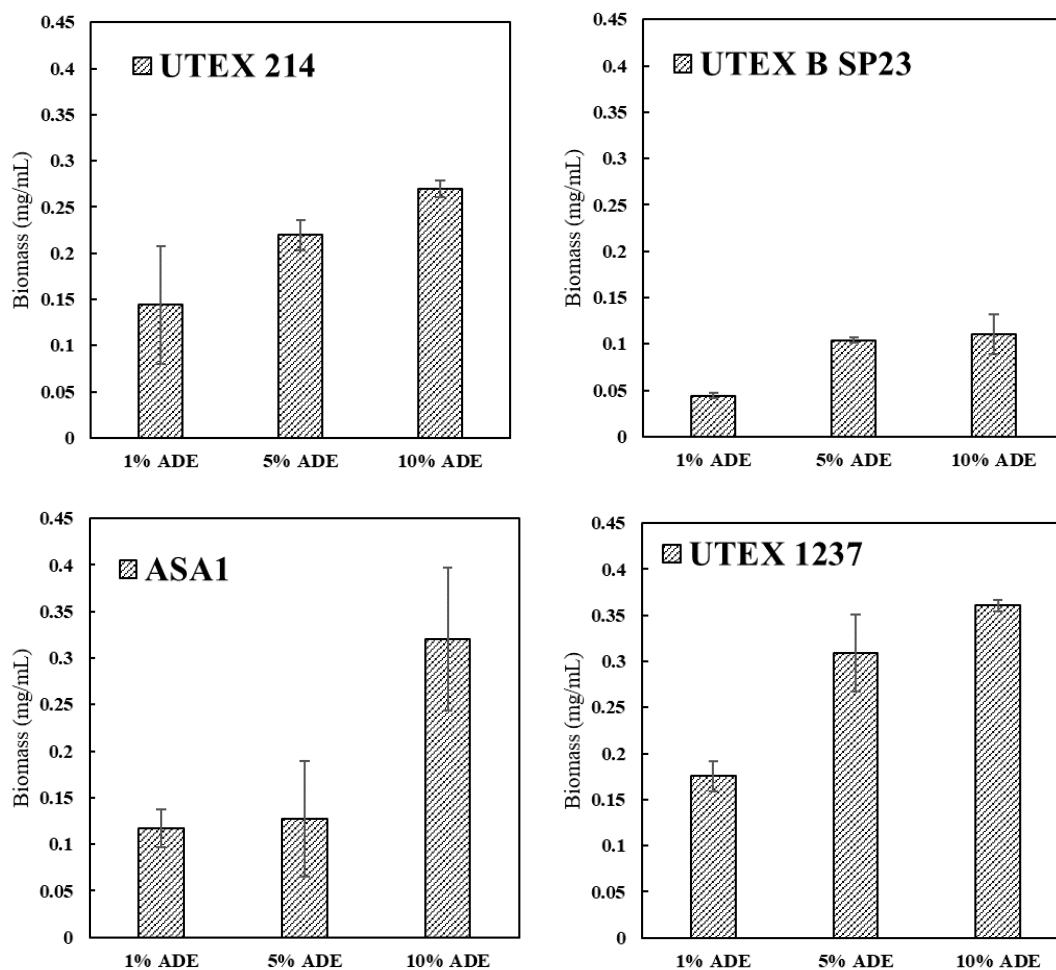


Figure 7. Biomass (mg/mL) of four strains under three different concentrations of ADE after freeze-drying. Error bars were calculated as the standard deviation of biological duplicates.

Nutrient recovery

Both N and P removal efficiency were measured before and after the growth experiments. Nitrogen recovery results are showed in Figures 8-10, and demonstrated as three separate parts, total nitrogen (TN), nitrate and ammonia. In Figure 8, UTEX 214 and UTEX SP23 have the highest nitrogen removal efficiency in 5% ADE, while ASA1 and UTEX 1237 have the highest nitrogen removal efficiency in 1% ADE. The removal efficiency of TN by UTEX 214 in three concentrations of ADE ranges from 47.9% to 88.4% while the efficiency of UTEX SP23 only ranges from 12.2% to 42.5%. Due to the detection limits of the ammonia Hach Kit (1-12 mg/L),

the ammonia concentrations of four strains that were cultivated in 1% ADE and UTEX 214 in 5% ADE are too low to be detected and not shown in Figure 10. According to Figure 10, the removal efficiency of ammonia is in the range of 34.8% to 59.2%. UTEX SP23 and UTEX 1237 have a slightly higher ammonia removal efficiency in 5% ADE than in 10% ADE, while ASA1 behaves the opposite way. Similar to ammonia, nitrate concentration of four strains in 1% ADE were below the limit of detection (0.23 mg/L as N). Interestingly, half of the available data of nitrate removal efficiency of algae strains we can get are negative and in the range of -41.4% to -6.84% indicating that nitrate was produced in some cases (Figure 8). In the meantime, positive removal efficiency is in the range of 0.58% to 26.5%.

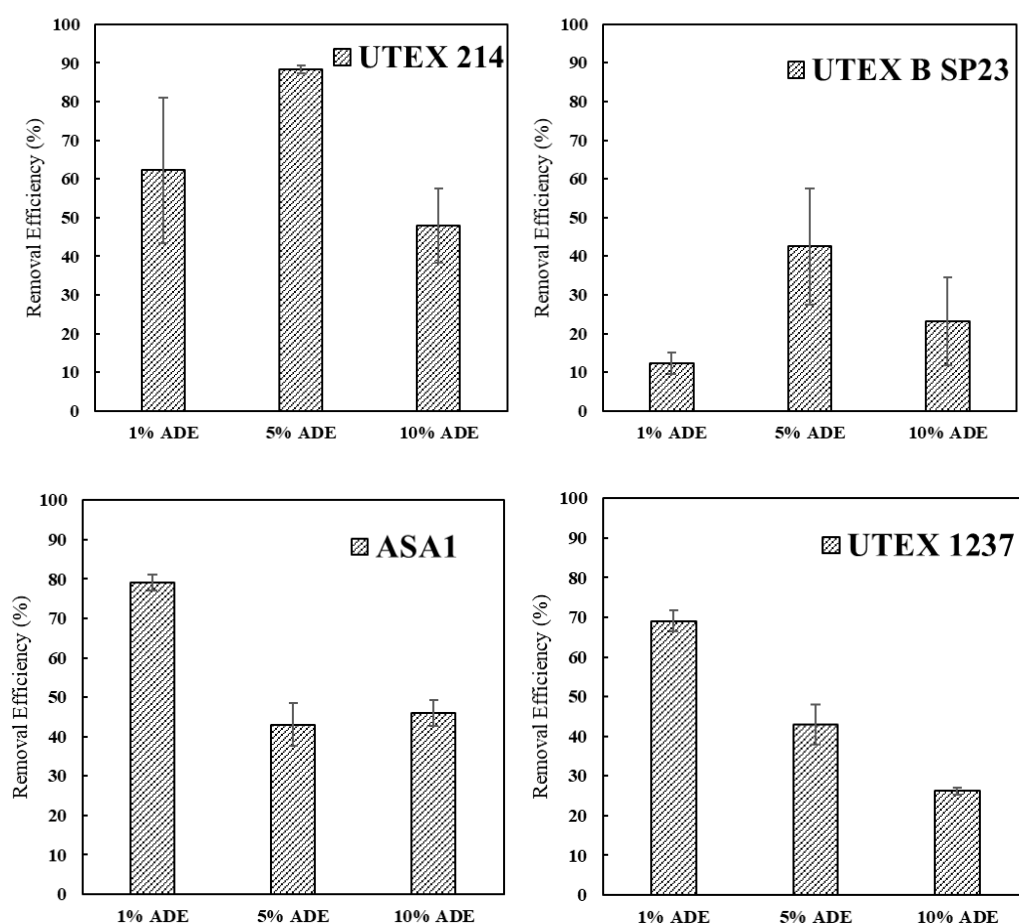


Figure 8. Total nitrogen removal efficiency of four strains in three different concentrations of ADE. Error bars were calculated as the standard deviation of biological duplicates.

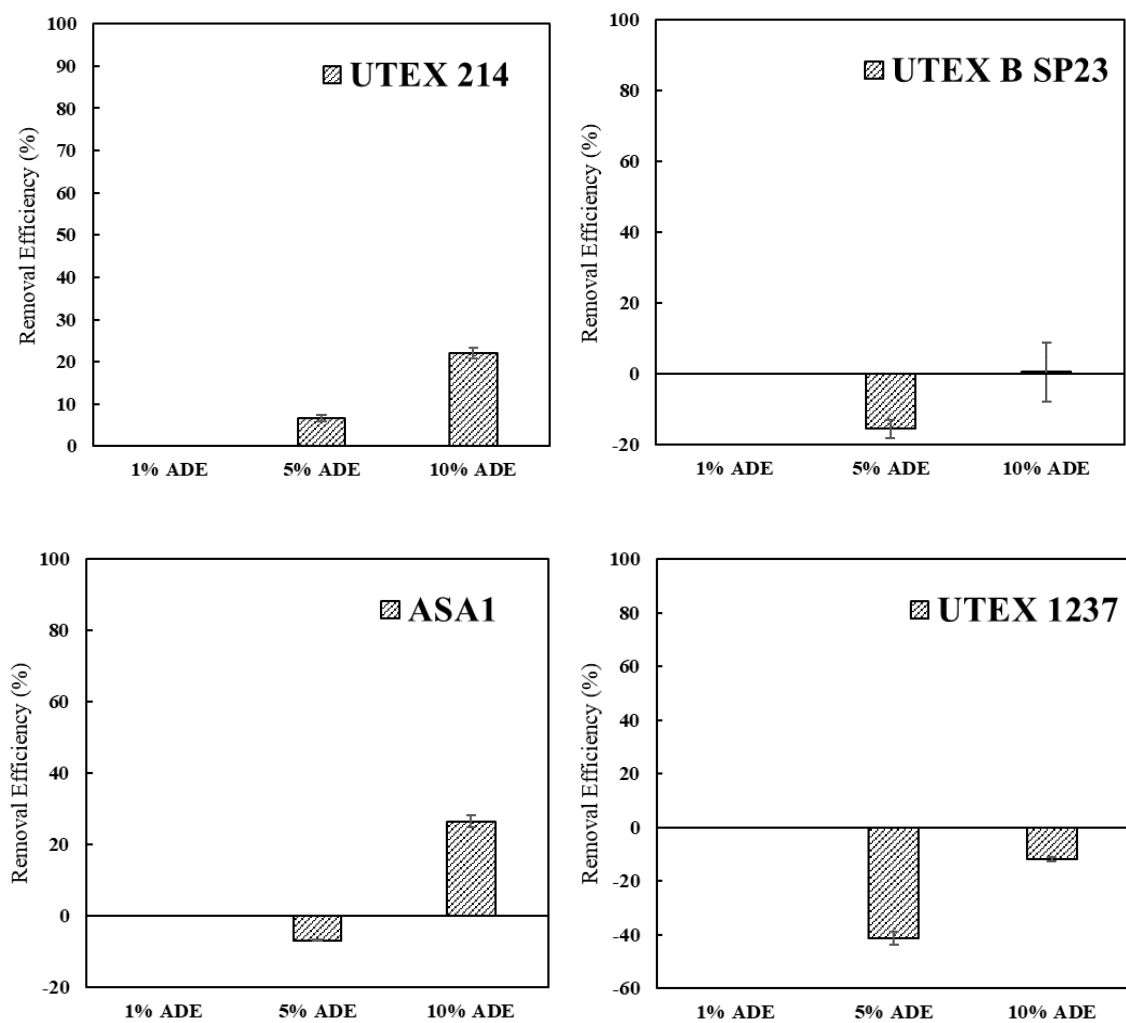


Figure 9. Total nitrate removal efficiency of four strains in two different concentrations of ADE. Error bars were calculated as the standard deviation of biological duplicates.

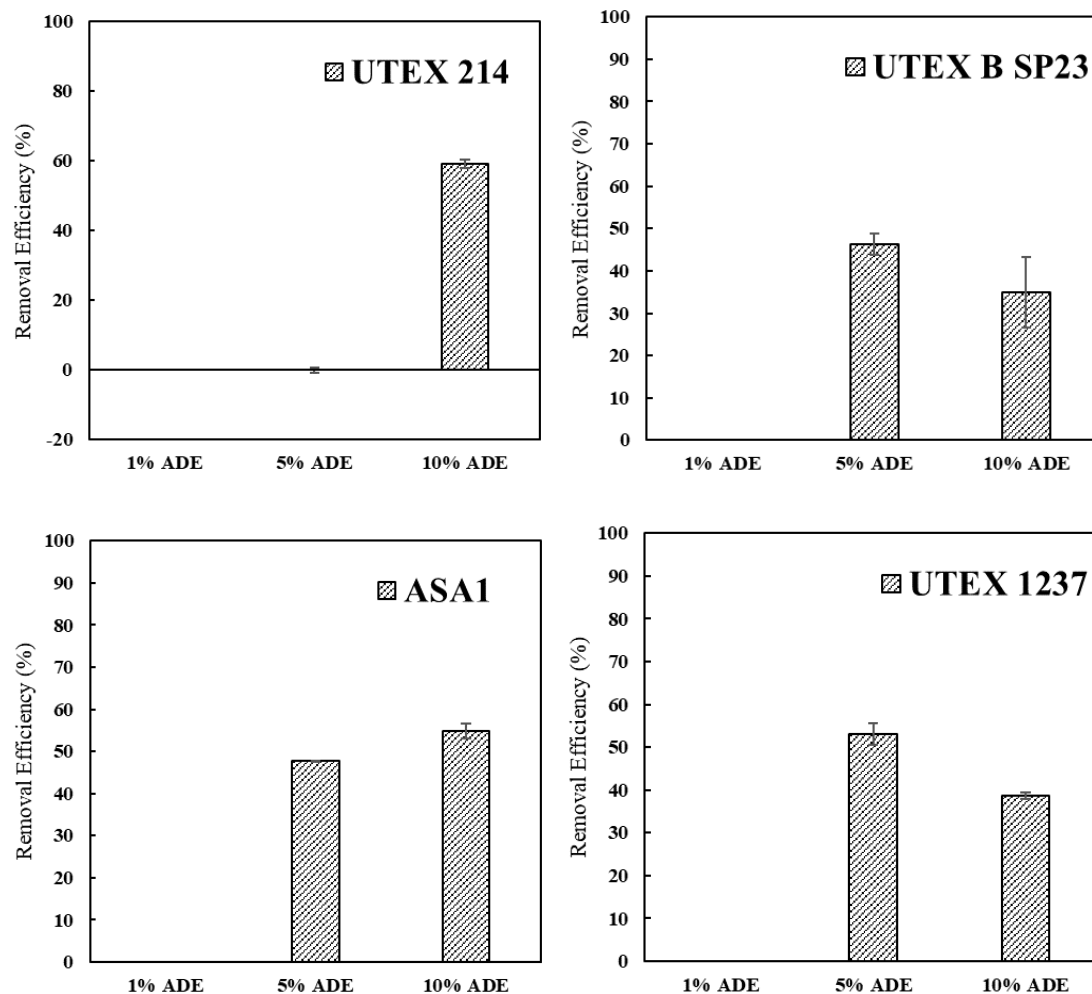


Figure 10. Total ammonia removal efficiency of three algae strains in two different concentrations of ADE and strain UTEX 214 in 10% ADE. Error bars were calculated as the standard deviation of biological duplicates.

Total phosphorus (TP) and orthophosphate were measured for each algae strain in three different concentrations of ADE, and the result of removal efficiency of TP is showed in Figure 11. Removal efficiency of orthophosphate in all three ADE concentrations and TP in 1% ADE are not shown because all of them are below the lowest limit of detection (0.15 mg/L as P). Based on Figure 10, UTEX 214, ASA1 and UTEX 1237 have a slightly better performance in 5% ADE than in 10% ADE, while UTEX SP23 performs better in 10% ADE. In addition, the removal efficiency is in the range of 65.2% to 72.3% in 5% ADE while in the range of 68.9%

to 78.1% in 10% ADE. And the difference of TP removal efficiency between each strain in 5% and 10% ADE are not as significant as within TN.

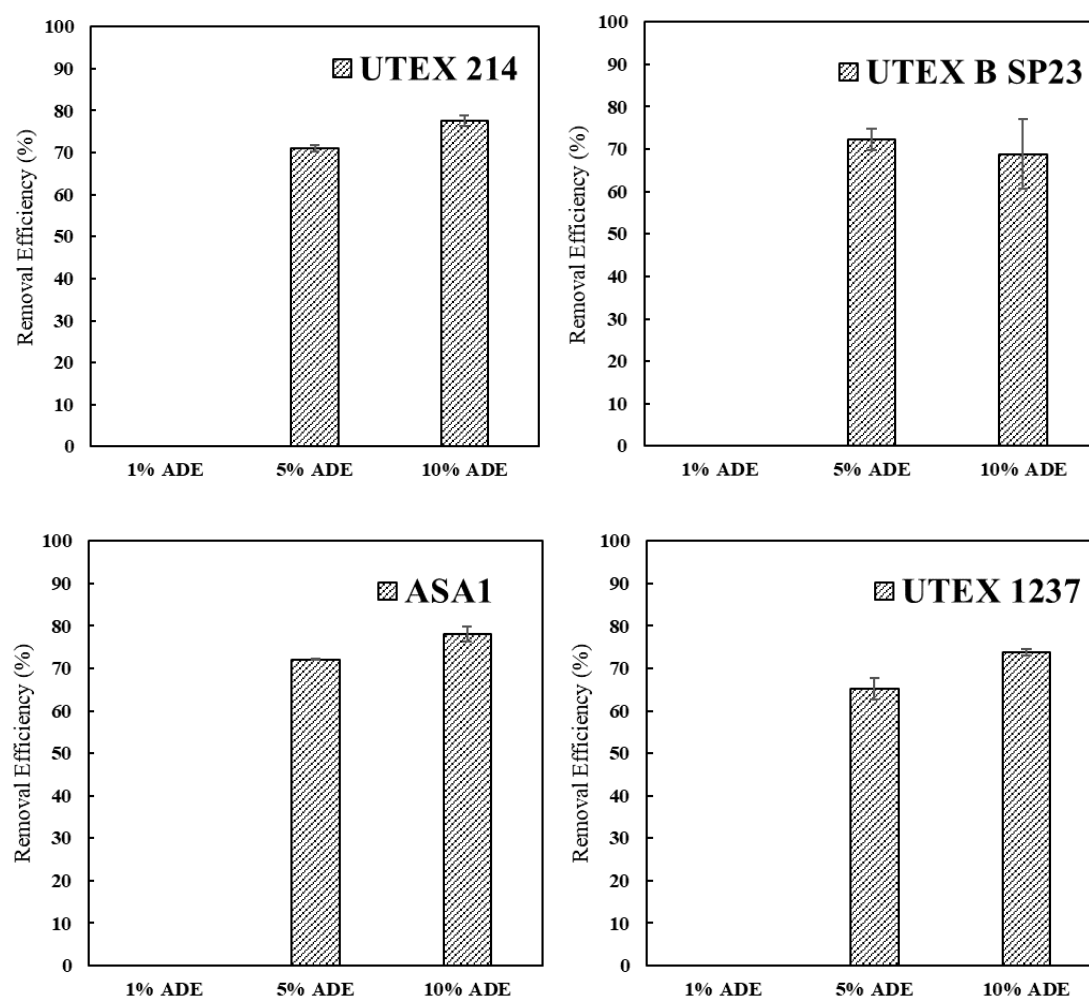


Figure 11. Total phosphorus removal efficiency of four strains in two different concentrations of ADE. Error bars were calculated as the standard deviation of biological duplicates.

Discussion

Screening

Dairy manure ADE was not centrifuged before adding it into the solution during the screening process, so there were large particles in the flasks that might influence both the light

intensity and initial nutrient concentration and thus lead to a possible difference the measured optical density and biomass. The result of 5% ADE was influence much more since there were more large particles introduced in the flasks. For example, there is a higher possibility to add large particles into columns and the participation of them will influence the optical density by interfering with the intensity of light. Besides, the ADE used in these experiments was collected from a lab-scale semi-continuous anaerobic digester that is subject to variability over time. Since samples were collected from different days, ADE that was used for diluting to 1% ADE and 5% ADE are different, leading to a degree of variability between experiments. In other words, 5% ADE tends to provide more organic compound and essential nutrients that can be used by microalgae (more than 5 times provided by 1% ADE) according to the data of the practical dairy manure organic loading rate of the laboratory-scale anaerobic digester. And this may be one of the reasons why most of the algae strains seems to have much higher densities and biomass in 5% ADE than 1% ADE. To be more specific, these extra organic compounds can be assimilated by certain algae strains and then used to promote their growth, thereby achieving efficient mixed nutrition and heterotrophic metabolism. In addition, as demonstrated by Figure 2A and 2B, algae strains that were cultivated in 5% ADE tend to have higher growth rate than 1% ADE. And this could also partially explain why 5% ADE might lead to a higher biomass production. Another reason might be that 5% ADE had a higher concentration of particles as habitation zones for mutually beneficial bacteria; the more particles the more bacteria present.

However, not every algae strain had the same phenomena. For example, UTEX 2532 and UTEX 2911 have higher density in 1% ADE instead of 5% ADE, algae strains like UTEX 74,

PE01, PEY basically only have slight differences between 1% ADE and 5% ADE during cultivation. And the reason might be 5% ADE has higher concentration of bacteria that can outcompete nutrient and organic carbon resources with algae strains. So even though there were high concentrations of nutrients and organic compounds, some of the algae strains that failed the competition with bacteria cannot take the best advantage of them. And this can also explain why some of the algae strains are less prolific than others when it comes to biomass production.

Selected strains cultivation

The ADE used to cultivate algae strains in this stage is a mixture of 7 days ADE samples that were collected from the laboratory anaerobic digestion reactor. By doing so, the characteristic of ADE that was used in each sample can be same. Besides, the ADE was also centrifuged before adding to samples which can prevent the interference caused by large particles such as fragmented straw. Based on the plots of optical density versus cultivation time, ASA1, UTEX SP23 and UTEX 1237 tend to have higher density with the increase of concentration of effluent, which followed the theory that algae strains can grow better with the higher concentration of nutrients that is provided by more ADE in the flasks. In addition, all four strains have the same pattern in biomass production that higher biomass corresponded to higher concentration of ADE due to the same reason. However, despite that ASA1 had higher optical density than UTEX 1237, UTEX 1237 actually produced more biomass, indicating that the cell density of UTEX 1237 might be larger than ASA1, as a result, the weight of biomass can be smaller even though there were much more cells. What's more, UTEX SP23 demonstrated a decline of optical density at the later period of cultivation, which reflected an

obvious existence of a death phase. In other words, UTEX SP23 has a shorter growth curve and would spend less time to go through the four phases of growth. As a result, the biomass production of this strain is the lowest at the end day of cultivation, and it is not valid to compare the biomass production of it with other algae strains since they are not at the same growth phase. Interestingly, the density of UTEX 214 in three different concentrations seems quite similar and did not change too much during cultivation time. And this can be explained by two different possibilities, one is that it has a relative long lag phase due to high concentration of effluent and 8 days of cultivation is too short for it to reach log phase, while the other possibility is that it had gone to death phase before we measured the optical density at the very first day. The second hypothesis seems to be more reliable if we take the relatively great biomass production and nutrient recovery result of UTEX 214 into consideration, besides, optical density at the first several days of it in 1% ADE showed a slight curve which might be evidence of decay. However, UTEX SP23 which also went through the death phase has a comparatively poor biomass production and nutrients recovery result and suggests that nutrients and organic compounds might be released from algal cells after it dies and undergoes decomposition. So, the possibility of UTEX 214 having a long lag phase still cannot be excluded yet.

Nutrient recovery

Most of the strains performed great in both total nitrogen and total phosphorus recovery, however, it seems that algae strains do not have a similar behavior in nutrients recovery, no matter nitrogen or phosphorus. Based on the results of nutrient recovery efficiency, UTEX 214 seems to be very impressive due to the contrast between optical density and nutrient recovery.

If UTEX 214 was still at lag phase at the end of cultivation, then the potential nutrient recovery of it can still be giant. From the plot, we could also find that the recovery efficiency of total nitrogen in 10% ADE was not better than in 5% ADE, and one possible reason is that no algae strains were at the stationary phase, which then lead to a difference in testing maximum nutrient recovery efficiency. Interestingly, the removal efficient of nitrate is really poor, some of the algae strains even have a negative efficiency whereas removal efficiency of ammonia is relatively good. One of the possible reasons would be that there exist some kind of bacteria that can actively transfer nitrate into ammonia. Another possible reason of why ammonia is a preferable source of nitrogen than nitrate is the less energy required for assimilation of ammonia since no electron donor is required. As for the recovery of phosphorus, there was no significant differences between each strain. Furthermore, although there was no plot of recovery efficiency of active phosphate since the concentration of all of the strains are below the lowest detection limit of the Hach Kit, it can still imply a great removal efficiency of active phosphate.

Conclusion

In conclusion, the behavior of each strains in ADE is highly variable. This work demonstrates that it is essential to explore which algae and cyanobacteria strains are more robust in unsterilized effluent based on their performance in both biomass production and nutrient recovery. Additionally, it is critically important to determine the most biologically and economically favorable concentration of ADE that can be used in industrial settings. The research in this paper only provides several possible combinations, and more experiments can

be done to fill this knowledge gap. To be more specific, the biomass production seems to increase with increasing concentrations of ADE. Additional experiments will help improve this combined biotechnology scheme for wastewater treatment and nutrient recovery.

Reference

Appendices

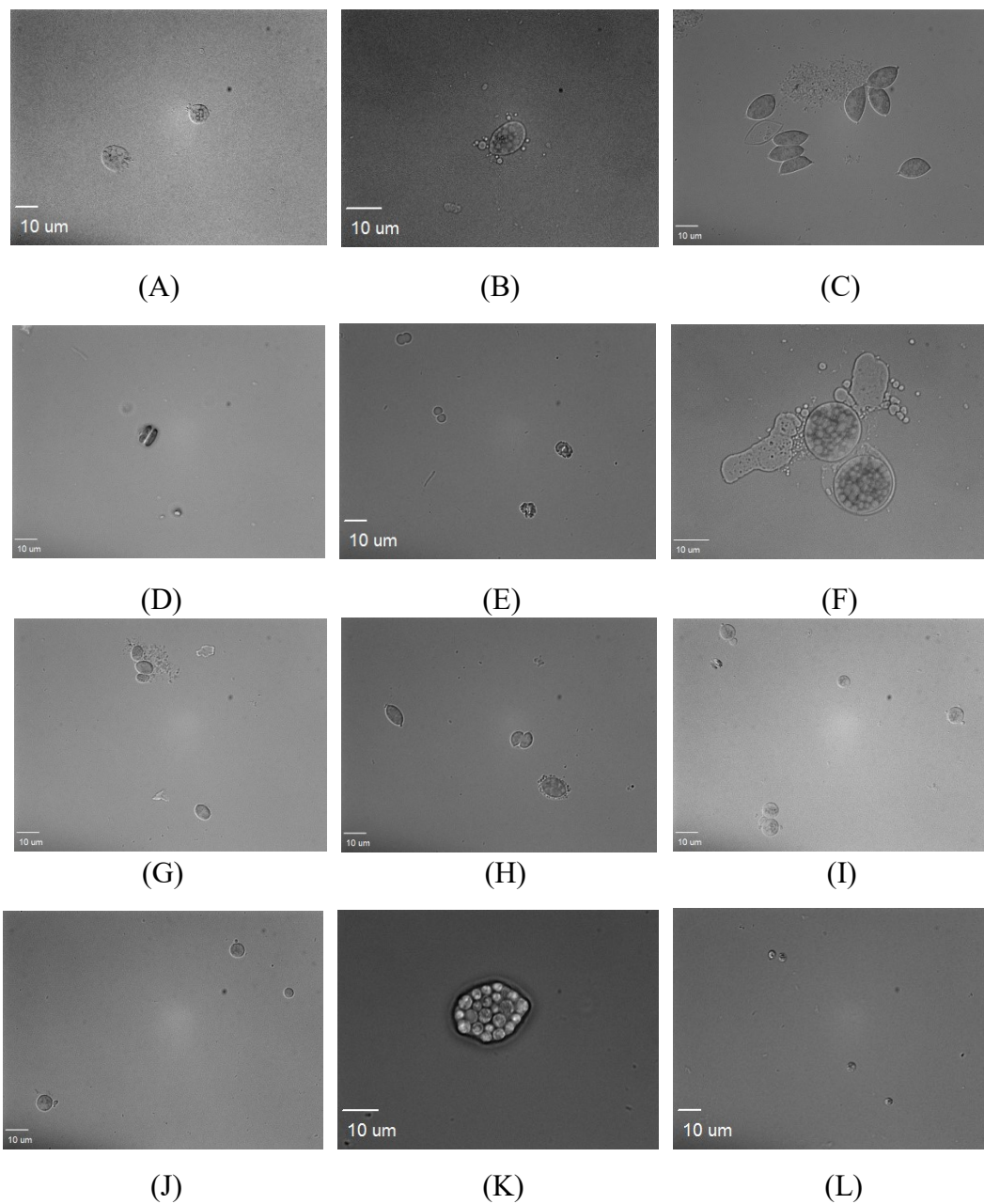


Figure S1. Images of selected strains under fluorescence microscope. (A) UTEX 260 (B) UTEX 969 (C) UTEX 1237 (D) UTEX 1584 (E) UTEX LB 2386 (F) UTEX 1907 (G) UTEX 2532 (H) UTEX 74 (I) PE-01 (J) PE-Y (K) AS-A1 (L) AS-A2

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Biography

Yuanyuan Zhao was born in 1995 in China.

She did her undergraduate work at Beijing University of Civil Engineering and Architecture, Beijing where she majored in Environmental Engineering. During her undergraduate study, she directed a large amount of time toward conducting research projects in order to master necessary techniques and gain insights. And based on coursework about sludge reduction, she came up with an idea about using *Myxomycetes* for residual sludge reduction and then worked on the project entitled Isolation and Purification of *Myxomycetes* and Their Application in Residual Sludge Reduction together with a partner since September 2015. Later in 2016, she finished her graduation project under the guidance of Assistant Professor Xiaoyan Zhang.

In 2017, Yuanyuan began her master's study at Johns Hopkins University.